

Regulation and roles of Ca²⁺ stores in human sperm

Correia, Joao; Michelangeli, Francesco; Publicover, Stephen

DOI:
[10.1530/REP-15-0102](https://doi.org/10.1530/REP-15-0102)

License:
Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Correia, J, Michelangeli, F & Publicover, S 2015, 'Regulation and roles of Ca²⁺ stores in human sperm', *Reproduction*, vol. 150, no. 2, pp. R65-R76. <https://doi.org/10.1530/REP-15-0102>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:
Eligibility for repository : checked 16/10/2015

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Regulation and roles of Ca^{2+} stores in human sperm

Joao Correia, Francesco Michelangeli and Stephen Publicover

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Correspondence should be addressed to S Publicover; Email: s.j.publicover@bham.ac.uk

Abstract

$[\text{Ca}^{2+}]_i$ signalling is a key regulatory mechanism in sperm function. In mammalian sperm the Ca^{2+} -permeable plasma membrane ion channel CatSper is central to $[\text{Ca}^{2+}]_i$ signalling, but there is good evidence that Ca^{2+} stored in intracellular organelles is also functionally important. Here we briefly review the current understanding of the diversity of Ca^{2+} stores and the mechanisms for the regulation of their activity. We then consider the evidence for the involvement of these stores in $[\text{Ca}^{2+}]_i$ signalling in mammalian (primarily human) sperm, the agonists that may activate these stores and their role in control of sperm function. Finally we consider the evidence that membrane Ca^{2+} channels and stored Ca^{2+} may play discrete roles in the regulation of sperm activities and propose a mechanism by which these different components of the sperm Ca^{2+} -signalling apparatus may interact to generate complex and spatially diverse $[\text{Ca}^{2+}]_i$ signals.

Reproduction (2015) **150** R65–R76

Ca^{2+} signalling in sperm

Cellular activity is constantly regulated by environmental cues and signals from other cells. Long-term regulation of cell function is normally achieved by control of gene expression, changing the complement and levels of proteins in the cell, but rapid or short-term changes are achieved by ‘post-translational’ protein modification, such as phosphorylation, sumoylation and nitrosylation, which alter the function/activity of proteins already present. Ca^{2+} -signalling is a key regulator of such post-translational modifications, with changes in cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) controlling the activities of key enzymes and proteins. Large changes in $[\text{Ca}^{2+}]_i$ can be achieved ‘instantaneously’ by flux of Ca^{2+} into the cytoplasm from the extracellular fluid or from storage organelles (primarily the endoplasmic reticulum) within the cell (Fig. 1a). The rapidity with which $[\text{Ca}^{2+}]_i$ -signals can be generated is crucial for ‘instantaneous’ cellular responses such as activation of muscle contraction and secretion of neurotransmitters that are achieved by rapid post-translational modification of protein function.

The highly condensed nucleus of sperm is transcriptionally silent (Miller *et al.* 2005, Miller & Ostermeier 2006) and translational activity is also negligible (though evidence has been presented for translation occurring at mitochondrial ribosomes; Gur & Breitbart 2008, Zhao *et al.* 2009, Chandrashekrana *et al.* 2014a,b). Regulation of sperm function is therefore dependent primarily on post-translational processes. $[\text{Ca}^{2+}]_i$ signalling is pivotal

to this regulation, and in mammalian sperm it plays a central role in controlling the cell’s behaviour (motility type and potentially chemotaxis), the induction of acrosome reaction (AR) and the process of capacitation (Publicover *et al.* 2007, Darszon *et al.* 2007, 2011). The importance for sperm function of membrane Ca^{2+} -channels and Ca^{2+} -influx is well established (Darszon *et al.* 2011) but there is also good evidence for the existence and functional importance of intracellular Ca^{2+} -storage organelles in sperm (Darszon *et al.* 2007, Publicover *et al.* 2007). Previously we reviewed the identities and functions of Ca^{2+} stores in sperm, focussing on the evidence for the existence of such stores, their components (pumps and channels) and their possible roles in the regulation of function in the mature sperm cell (Costello *et al.* 2009). Since then considerable progress has been made in understanding the central role of Ca^{2+} signalling in the regulation of mammalian and non-mammalian sperm function and the mechanisms by which sperm $[\text{Ca}^{2+}]_i$ signals are generated. In particular successful application of whole cell patch clamp technique, in human as well as mouse sperm, has revealed the central importance of Ca^{2+} influx through CatSper, a sperm specific, Ca^{2+} -permeable channel in the membrane of the flagellar principal piece. Male mice null for CatSper are infertile (Ren *et al.* 2001) and their sperm show defective motility (Carlson *et al.* 2003). Here we review recent progress in understanding the diversity of mechanisms for the regulation of Ca^{2+} store activity and the evidence for their involvement in controlling sperm function.



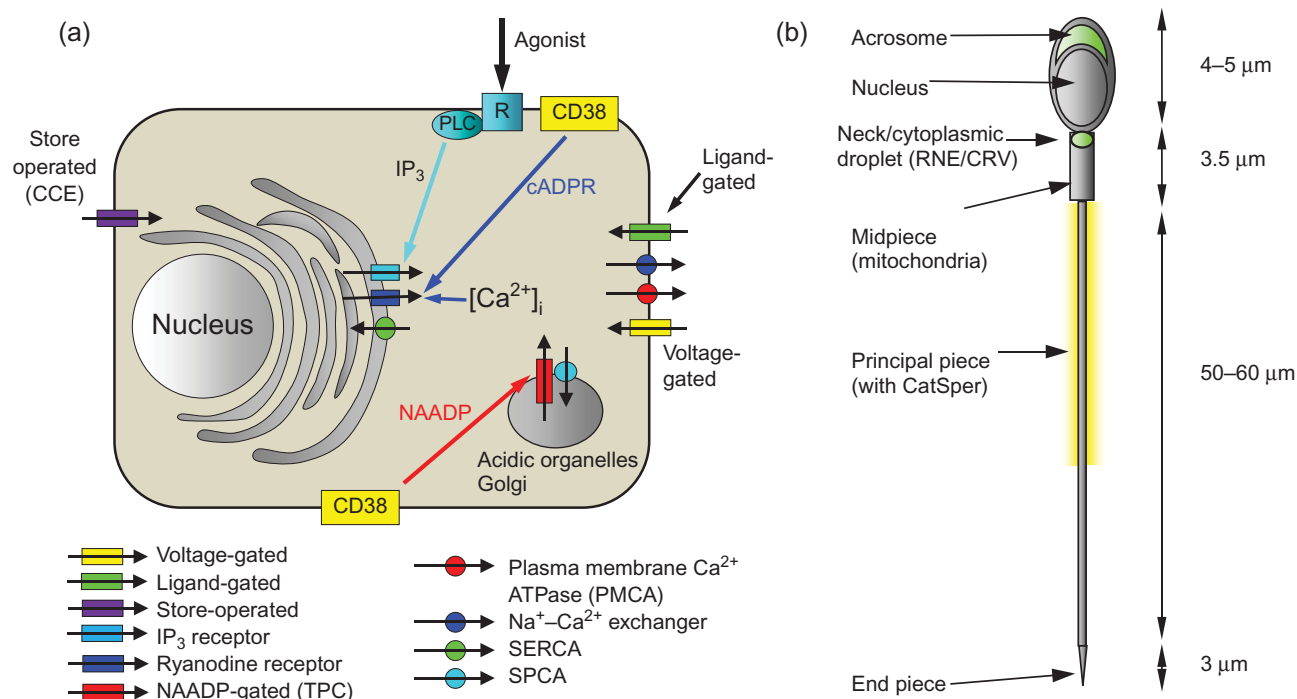


Figure 1 (a) Simplified diagrammatic summary of $[Ca^{2+}]_i$ signalling toolkit in a somatic cell. Ion channels are shown as rectangles with arrow indicating normal direction of Ca^{2+} flow (yellow, voltage-gated; green, ligand-gated; purple, store-operated; light blue, IP₃ receptor; dark blue, ryanodine receptor; red, NAADP-gated). Pumps are shown as circles with arrows indicating normal direction of Ca^{2+} movement (red, PMCA; blue, Na⁺-Ca²⁺ exchanger; green, SERCA; light blue, SPCA). Activation of IP₃ receptors by membrane receptor activation and phospholipase C is shown in light blue. Generation of cADPR and NAADP by CD38 and possibly other enzymes (leading to mobilisation of Ca^{2+} from intracellular stores) is shown by yellow boxes. (b) Structure of human sperm showing positions of CatSper channels (yellow shading around anterior flagellum) and Ca^{2+} stores in the acrosome and at the sperm neck (redundant nuclear envelope and calreticulin-containing vesicles) (shown in green).

Ca²⁺ stores and their regulation

The importance of Ca^{2+} stores in generating complex Ca^{2+} signals in somatic cells has long been recognized. Until relatively recently the endoplasmic reticulum Ca^{2+} store has been the major focus for research as this was the first organelle to show controllable mobilization of Ca^{2+} through second messengers acting upon intracellular Ca^{2+} channels, as well as being able to be refilled via Ca^{2+} pumps. Additionally, these Ca^{2+} signals could also be re-modelled through the regulation of these Ca^{2+} transporters to generate complex spatial and temporal Ca^{2+} transients (Berridge *et al.* 2003). It has now become clear that many other organelles such as mitochondria, endosomes, lysosomes and Golgi complexes also contribute to the generation and propagation of these complex Ca^{2+} signals within cells (Michelangeli *et al.* 2005). Furthermore, novel Ca^{2+} transporters have also been identified within these other organelles and several have recently been identified in sperm (Costello *et al.* 2009).

Intracellular Ca²⁺ channels

The major intracellular Ca^{2+} channels that have been identified and appear to be almost ubiquitously distributed within mammalian cells, especially on the

endoplasmic reticulum, include the inositol-1,4,5-trisphosphate-(IP₃)-sensitive Ca^{2+} channel (or IP₃ receptor; IP₃R) and the ryanodine receptor (RyR) (Michelangeli *et al.* 2005) (Fig. 1a). The IP₃ receptor, as the name implies, is activated by the second messenger IP₃ that is generated through the hydrolysis of phosphatidylinositol-4,5-bisphosphate. This channel has a specific IP₃ binding site that is located towards the N-terminus of the protein (Seo *et al.* 2012) and also has a requirement for Ca^{2+} which acts as a co-agonist in order for the channel to open (Bezprozvanny *et al.* 1991). The activation of RyR is likely to be through a mechanism involving Ca^{2+} induced Ca^{2+} release (CICR) and by the action of the putative second messenger cyclic-adenosine diphospho-ribose (cADPR) (Ogunbayo *et al.* 2011) (Fig. 1a). cADPR is made from NAD by the action of an ADP-ribosyl cyclase enzyme such as CD38 (Cosker *et al.* 2010), although other as yet unidentified enzymes may also be involved in catalysing this reaction (Guse 2015). It is as yet unclear whether, unlike the IP₃R, cADPR binds directly to RyR or whether it binds to accessory proteins such as calmodulin or FK506-binding protein, that then interact with the RyR (Guse 2015).

Another metabolite of NAD which is believed to have Ca^{2+} mobilizing ability is nicotinic acid adenine dinucleotide phosphate (NAADP; Genazzani *et al.* 1997).

NAADP is made from NADP through the action of either CD38 acting as a base-exchanger, swapping the nicotinamide group for nicotinic acid or via an unidentified NADP-deaminase (Guse 2015). NAADP is believed specifically to mobilize Ca^{2+} from acidic stores such as lysosomes (Churchill *et al.* 2002, Menteyne *et al.* 2006), which can then induce CICR at RyRs and IP_3 Rs in mammalian cells (Cancela *et al.* 1999; Fig. 1a). Results initially presented by Calcraft *et al.* (2009), indicated that NAADP specifically activates Ca^{2+} -specific two-pore channels (TPC) within the acidic organelles, these channels being first described in plants (Peiter *et al.* 2005). However, in kinetic studies there is a prominent lag between addition of NAADP and Ca^{2+} mobilization (Genazzani *et al.* 1997). Combined with the observation that photo-affinity labelling with azido-NAADP (Lin-Moshier *et al.* 2012) showed labelling of only low molecular weight proteins, not consistent with TPCs, it suggests that NAADP might function by binding to accessory proteins rather than directly to the channel. Recently there has been considerable controversy as to whether the NAADP-sensitive Ca^{2+} channel is a TPC (Morgan & Galione 2014). Data from two studies (Wang *et al.* 2012, Cang *et al.* 2013) suggested that TPCs are in fact Na^+ -specific channels with very low Ca^{2+} selectivity that are activated by phosphoinositide lipids and modulated by mTOR, but not by NAADP. However, recently published work with cells from mice null for TPC1 and TPC2 provided strong evidence that TPCs are similarly permeable to Ca^{2+} and Na^+ and are NAADP-gated through binding to an accessory protein (Ruas *et al.* 2015).

Numerous kinases have been shown to modulate the activity of both the IP_3 Rs and RyRs, including several ubiquitous ser/thr kinases such as PKA, PKG and CaMKII (Yule *et al.* 2010, Camors & Valdivia 2014). Indeed, some of these kinases such as PKA appear to have both stimulatory and inhibitory effects on the IP_3 R, dependent upon isoform subtype and the presence of multiple kinase-dependent phosphorylation sites on the same receptor (Dyer *et al.* 2003). Less ubiquitous ser/thr kinases such as Akt and polo kinases as well as tyrosine kinases such as fyn kinase have also been shown to affect these channels (Yule *et al.* 2010, Camors & Valdivia 2014).

Both the RyRs and the IP_3 Rs are modulated by changes in their oxidation states caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), and this occurs mainly through modification of specific cysteine (cys) amino acid residues. Oxidation of these cys residues in RyRs occurs both by S-glutathionylation as well as S-nitrosylation by the second messenger nitric oxide (NO; Csordas & Hajnoczky 2009) and promotes the activity of the channel by enhancing RyR subunit interactions and also by reducing the efficacy of inhibitory modulators (Hamilton & Reid 2000). In IP_3 Rs the effects of oxidative stress are complex: low levels

of cys oxidation caused by low concentrations of thimerosal (a cys-modifying mercuric compound) and naturally generated ROS cause sensitization of this channel, while higher concentrations of thimerosal inhibit channel activity (Missiaen *et al.* 1991, Sayers *et al.* 1993). Currently, however, there is little evidence that NO can affect the activity of the IP_3 Rs.

Intracellular Ca^{2+} pumps

The major transporter involved in refilling Ca^{2+} stores within the endoplasmic reticulum is the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA; Fig. 1a), and these pumps occur abundantly in all somatic cells. Their role is to pump Ca^{2+} back into the storage organelles to help terminate Ca^{2+} signals (Michelangeli *et al.* 2005, Michelangeli & East 2011). There are three isoforms of this Ca^{2+} ATPase, each encoded by a different gene and each isoform can exist in a variety of spliced variants that differ in size and regulatory properties (Michelangeli & East 2011). SERCA1 is mainly confined to skeletal muscle, while SERCA2 is widely distributed in most other tissues and organs and type 3 has a limited expression. Another related Ca^{2+} ATPase that is also found ubiquitously within somatic cells is the secretory pathway Ca^{2+} ATPase (SPCA), which is localized to the Golgi apparatus (Wootton *et al.* 2004). SPCA exists in two isoforms with the expression of type 1 being far more widespread than type 2, which appears to be mainly located within glandular tissues (Vanoevelen *et al.* 2005). Recently there has been evidence to suggest that SPCA2 can interact with and regulate the plasma membrane located ORAI Ca^{2+} channels that are implicated in store-operated Ca^{2+} entry (Feng *et al.* 2010), which may indicate a dual function for this Ca^{2+} ATPase in cells that express it.

There is currently some debate as to which type of intracellular Ca^{2+} ATPase is expressed in mature sperm. We have highlighted that SPCA1 is present in human sperm, where it appears to be mainly localized to the neck region of the cell where the redundant nuclear envelope (RNE) and calreticulin-containing vesicles are situated (Harper *et al.* 2005). This study also found no evidence for expression of SERCA in human sperm as no cross-reactivity was observed with a pan-isoform SERCA antibody and no effects on $[\text{Ca}^{2+}]_i$ were observed with specific but saturating concentrations of the SERCA-inhibitor thapsigargin. However, a more recent study (Lawson *et al.* 2007) detected SERCA2, mainly localized to the acrosome and mid-piece, using a SERCA2-specific antibody.

Unlike the intracellular Ca^{2+} channels, there is no strong evidence to suggest that either SERCA or SPCA can be directly phosphorylated and regulated by protein kinases, although some Ca^{2+} ATPase modulatory proteins like phospholamban (that is found almost exclusively in heart) are regulated through

phosphorylation by PKA, PKG and CamKII (Colyer 1998). There is considerable evidence indicating that oxidative stress can modulate SERCA activity (although no studies have yet been undertaken on SPCA). Again a number of critical cyst residues such as cyst674 can be S-glutathionylated to cause an increase in SERCA pump activity (Adachi *et al.* 2004). Modifications of other cyst residues on the Ca^{2+} ATPase, however, can have inhibitory effects (Sayers *et al.* 1993, Sharov *et al.* 2006, Csordas & Hajnoczky 2009).

Ca^{2+} stores, mechanisms for store mobilisation and store-operated Ca^{2+} channels in sperm

During the later stages of their development spermatozoa shed much of their cytoplasm including intracellular organelles. Thus mammalian sperm contain no organised endoplasmic reticulum. However, studies on the expression of Ca^{2+} store components and on the generation $[\text{Ca}^{2+}]_i$ signals suggest that the remaining intracellular organelles function as Ca^{2+} -stores and play a significant role in the regulation of cellular function (Costello *et al.* 2009). In particular, the acrosomal vesicle at the apex of the head and the collection of vesicular membranous structures that occur at the sperm neck and anterior midpiece (including the cytoplasmic droplet of human sperm) appear to be functionally important Ca^{2+} -stores (Fig. 1b; shown in green). At both these locations IP_3Rs have been detected in human and in bovine sperm by immuno-staining (Dragileva *et al.* 1999, Kuroda *et al.* 1999, Ho & Suarez 2001, 2003, Naaby-Hansen *et al.* 2001). RyRs have also been detected in human and rodent sperm (Trevino *et al.* 1998, Lefievre *et al.* 2007). Staining of human sperm with anti-RyR1, anti-RyR2, pan-RyR and BODIPY-FLX ryanodine is localised primarily to the neck region, though some acrosomal staining was also observed (Harper *et al.* 2004, Lefievre *et al.* 2007, Park *et al.* 2011). In contrast, other authors (Ho & Suarez 2001) have reported no staining of bovine sperm with BODIPY-FLX ryanodine (see Costello *et al.* (2009) for further discussion). Thus mobilisation of stored Ca^{2+} in mammalian sperm may occur in response to generation of IP_3 by activity of phospholipase C and by CICR at IP_3Rs or RyRs. These processes can be sensitised by effects such as oxidative stress and S-nitrosylation (see ' Ca^{2+} stores and their regulation'). For instance, exposure of human sperm to $\text{NO}\cdot$ at levels equivalent to those produced by explants of reproductive tract lining mobilises stored Ca^{2+} and modifies flagellar activity (Lefievre *et al.* 2007, Machado-Oliveira *et al.* 2008).

In addition to generation of IP_3 in sperm, there is evidence that other Ca^{2+} mobilising messengers (NAADP and cADPR) are synthesised in sperm and/or produced in response to stimulation. Sea urchin sperm contain significant levels of both cADPR and NAADP,

which may contribute to oocyte activation (Chini *et al.* 1997, Billington *et al.* 2002). Human sperm have been shown to contain cADPR at micromolar concentrations but NAADP was not detected (Billington *et al.* 2006). Interestingly, this study also demonstrated synthesis of cADPR by human sperm but the ecto-enzyme CD38 (an enzyme present on mammalian cells that synthesises both cADPR and NAADP; see ' Ca^{2+} stores and their regulation') could not be detected by western blotting. In contrast, Park *et al.* (2011), reported detection of CD38 in human sperm after co-incubation with prostasomes (prostate-derived membrane vesicles; see below). Furthermore, the presence of a novel NAADP synthase, which lacks the cyclase activity of CD38, has been described both in sea urchin (Vasudevan *et al.* 2008) and human sperm (Sanchez-Tusie *et al.* 2014). In sea urchin sperm this enzyme is strongly Ca^{2+} -regulated and most active at acid pH whereas the human enzyme shows only weak Ca^{2+} -regulation and activity is maximal at pH 7–8 (Vasudevan *et al.* 2008, Sanchez-Tusie *et al.* 2014).

Recent findings have supported the idea that NAADP is functional in human sperm. Sanchez-Tusie *et al.* (2014) investigated the effects of cell-permeant (AM-ester) derivatives of NAADP and cADPR. No effects were observed with cADPR, consistent with previous pharmacological investigation by Billington *et al.* (2006), but NAADP caused elevation of $[\text{Ca}^{2+}]_i$ both in cells incubated under standard conditions and also when $[\text{Ca}^{2+}]_o$ was buffered to 100 nM, conditions under which Ca^{2+} influx is negligible and $[\text{Ca}^{2+}]_i$ signalling depends solely on mobilisation of stored Ca^{2+} . Staining of NAADP receptors using the fluorescent NAADP receptor ligand Ned-19 and identification of acidic organelles using lysotracker highlighted both an anterior store (potentially the acrosome) and a store at the sperm neck (Fig. 1b). Consistent with these findings, Arndt *et al.* (2014), studying AR (see below), provided evidence for involvement in this process of NAADP and TPCs, which have been proposed to be the NAADP receptor/ Ca^{2+} channel of acidic Ca^{2+} storage organelles (Calcraft *et al.* 2009; Fig 1a; see ' Ca^{2+} stores and their regulation').

Park *et al.* (2011) investigated the incorporation into human sperm of proteins from prostasomes (prostate-derived vesicles which are normally added to sperm during ejaculation) and their effects on $[\text{Ca}^{2+}]_i$ signalling. They concluded that CatSper channel proteins were present in the differentiated sperm, but other Ca^{2+} signalling 'tools' including RyRs and CD38 were added to the freshly-ejaculated sperm upon mixing with prostasomes, by fusion with the membrane of the midpiece. They also examined the effects of stimulation with progesterone on $[\text{Ca}^{2+}]_i$ and motility of sperm exposed to prostasomes and sperm that had been rapidly removed from semen to minimise mixing with prostasomes. Their data suggest that the generation of

sustained $[\text{Ca}^{2+}]_i$ signals (such as the second component of the biphasic progesterone-induced $[\text{Ca}^{2+}]_i$ signal) and consequent effects on motility may depend, at least partly, upon generation of cADPR by prostates-derived enzymes. Interestingly, CD38-null mice proved to be fertile, but analysis showed that 20% of normal ADPR cyclase activity remained in prostates from these animals, indicating the presence of a non-CD38 ADPR-cyclase, potentially that described by Sanchez-Tusie *et al.* (2014). Thus both NAADP and cADPR are potentially synthesised by sperm and involved in regulation of sperm Ca^{2+} store activity but their roles are not yet clear.

In somatic cells mobilisation of stored Ca^{2+} induces secondary Ca^{2+} influx through channels at the cell membrane (store-operated channels, SOCs) by the process of capacitative Ca^{2+} entry (CCE) (Fig. 1a). CCE both prolongs Ca^{2+} signals that are induced by store mobilisation and provides Ca^{2+} for re-charging of the storage organelles. Recently great progress has been made in elucidating the key players and mechanisms in this process. Stromal interaction molecule (STIM) has been identified as the sensor molecule present in the membrane of the Ca^{2+} store. The intraluminal part of STIM includes a Ca^{2+} -binding EF hand that detects depletion of stored Ca^{2+} . STIM then redistributes, moving to a position adjacent to the plasma membrane where it activates channel proteins (ORAI and possibly members of the TRPC (transient receptor potential canonical) family (Cahalan 2009)). $[\text{Ca}^{2+}]_i$ signals in human and other mammalian sperm induced by agonists and by treatments designed to mobilise stored- Ca^{2+} show characteristics consistent with the occurrence of CCE (Blackmore 1993, Dragileva *et al.* 1999, O'Toole *et al.* 2000, Park *et al.* 2011, Lefievre *et al.* 2012). STIM1, ORAI and TRPC proteins have been detected in human sperm (Castellano *et al.* 2003, Darszon *et al.* 2012, Lefievre *et al.* 2012), STIM1 being localised primarily to the neck region/midpiece and the acrosome where Ca^{2+} stores are present (Lefievre *et al.* 2012). To date the application of whole-cell patch clamp has not provided evidence for the occurrence of CCE in human sperm (Lefievre *et al.* 2012) so these findings must be interpreted cautiously, but $[\text{Ca}^{2+}]_i$ signals generated by mobilisation of Ca^{2+} stores in sperm may be amplified by activation of CCE. Induction of CCE in somatic cells can have a latency of tens of seconds due to the need for STIM to migrate to the peripheral portions of the endoplasmic reticulum where it can interact with SOC proteins (Luik *et al.* 2006, Wu *et al.* 2006), but in sperm the storage organelles are close to the plasma membrane and STIM proteins are localised here, such that CCE could be near 'instantaneous'. Pre-treatment of human sperm with low concentrations of 2-aminoethoxydiphenyl borate, which potentiates CCE by promoting the interaction of STIM with SOCs (Navarro-Borelly *et al.* 2008, Wang *et al.* 2009,

Yamashita *et al.* 2011) significantly enhanced the amplitude of the progesterone-induced Ca^{2+} transient at the sperm neck (where secondary release of stored Ca^{2+} may occur; Fig. 1b; see 'Model for interaction of CatSper channels and Ca^{2+} -stores') but did not affect the response in the flagellum, where progesterone activates CatSper channels (Fig. 1b), or the kinetics of the signal at either location (Lefievre *et al.* 2012). Conversely, when sperm were pre-treated with a cell-penetrating peptide that mimics part of the key SOAR region of STIM1 (potentially preventing auto-inhibitory folding of STIM upon store-refilling) there was a marked prolongation of the progesterone-induced $[\text{Ca}^{2+}]_i$ transient in a subset of cells (Morris *et al.* 2015).

Mobilisation of sperm Ca^{2+} stores by agonists

In the majority of somatic cells mobilisation of stored Ca^{2+} occurs upon agonist-induced synthesis of Ca^{2+} mobilising intracellular messengers. Thus agonist-induced synthesis of inositol trisphosphate, cADPR and NAADP can lead to rapid release of stored Ca^{2+} and generation of local, global and complex spatio-temporal signals (Fig. 1a). Is there evidence that such processes occur and are functionally significant in responses to agonist stimulation of sperm?

The best-characterised agonist-induced $[\text{Ca}^{2+}]_i$ signals in sperm are responses to solubilised zona pellucida/zona proteins in mouse cells and progesterone in human. Application of patch clamp has clearly shown that the primary action of progesterone in human sperm is to activate CatSper channels, leading to Ca^{2+} -influx (Lishko *et al.* 2011, Strunker *et al.* 2011). Strunker *et al.* (2011) investigated the $[\text{Ca}^{2+}]_o$ dependence of progesterone-induced $[\text{Ca}^{2+}]_i$ signals in rapid-mixing experiments on human sperm and reported that buffering of $[\text{Ca}^{2+}]_o$ to ≤ 100 nM abolished the response (though see Espino *et al.* (2009)), suggesting that any mobilisation of stored Ca^{2+} is a secondary response. Synthesis of IP_3 is reported to occur downstream of progesterone-induced Ca^{2+} influx (Thomas & Meizel 1989), an important observation that should be pursued. Stimulation of mouse sperm with zona proteins induces AR, which requires elevation of $[\text{Ca}^{2+}]_i$ in the sperm head (Florman *et al.* 2008) and is dependent on mobilisation of Ca^{2+} from the acrosomal store (De Blas *et al.* 2002; see below). The nature of the Ca^{2+} influx following stimulation is not clear and several channels may be involved (Florman *et al.* 2008, Xia & Ren 2009, Cohen *et al.* 2014), but Ca^{2+} signals are sensitive to inhibition of G-protein signalling (using pertussis toxin) and inhibition of PLC (Florman *et al.* 2008, Ren & Xia 2010). Furthermore, in sperm from mice null for PLC β 4 (in which males' fertility is severely impaired) the $[\text{Ca}^{2+}]_i$ response is reduced and zona-induced AR does not occur (Fukami *et al.* 2001, 2003). Thus conventional IP_3 -induced mobilisation of stored Ca^{2+}

is apparently central to this essential aspect of mammalian sperm physiology.

Evidence for the existence of other store-mobilising agonists is largely preliminary, but there are a number of candidates, of which the best-studied is vitamin D (Blomberg Jensen 2014). Human sperm have been shown to express vitamin D receptor (VDR; Aquila *et al.* 2009, Blomberg Jensen *et al.* 2010, 2011), the enzymes CYP27B (which produce the active compound (1,25(OH)₂D₃) cholecalciferol) and the inactivating enzyme CYP24A1 (Blomberg Jensen *et al.* 2010, 2011). All are expressed in the neck region of the sperm and staining of cells for VDR and CYP24A1 shows a strong association. In sub-fertile patients the proportion of cells expressing CYP24A1 varies greatly and is significantly correlated with semen quality (sperm count, concentration, morphology and motility; Blomberg Jensen *et al.* 2011, 2012). Stimulation of human sperm with 1,25(OH)₂D₃ (100 pM–1 µM) induced a [Ca²⁺]_i response, including a transient and plateau, that was blocked by pre-treatment with the non-genomic VDR antagonist 1β,25(OH)₂D₃ but was insensitive to the nuclear VDR antagonist ZK159222 (Blomberg Jensen *et al.* 2011). This effect was greatly reduced by pre-treatment with the phospholipase C inhibitor U73122 (2 µM) but was also inhibited by incubation in EGTA-buffered medium for up to 20 min prior to stimulation. Both motility and AR were significantly increased upon stimulation with 1,25(OH)₂D₃ (Blomberg Jensen *et al.* 2011).

Kisspeptin, a peptide agonist of the G-protein coupled receptor GPR54/KISS1R, has also been shown to cause sustained, dose-dependent elevation of [Ca²⁺]_i in human and in mouse sperm (Pinto *et al.* 2012, Hsu *et al.* 2014). In neurons binding of kisspeptin to its receptor activates PLC and results in generation of IP₃ and diacylglycerol, leading to mobilisation of stored Ca²⁺ and also depolarisation (Liu *et al.* 2008, Pielecka-Fortuna *et al.* 2008, Beltramo *et al.* 2014). In human sperm the effect of kisspeptin on [Ca²⁺]_i did not occlude the response to stimulation with the CatSper agonist progesterone and was not reduced when applied in the presence of progesterone (Pinto *et al.* 2012). Both KISS1R and kisspeptin itself were detected in the head of human sperm, suggesting that an autocrine action of the peptide may occur. Motility parameters of kisspeptin-treated cells were significantly altered, including an increase in lateral movement of the head and a decrease in linearity of the sperm path, characteristics of hyperactivated sperm (Pinto *et al.* 2012). Ghrelin, another peptide hormone that also acts through mobilisation of stored Ca²⁺ (Camina *et al.* 2003), has also been detected in human sperm (Moretti *et al.* 2014). Micromolar concentrations of ghrelin have been shown to increase [Ca²⁺]_i and motility in rat sperm (Lukaszyk *et al.* 2012), but expression of ghrelin receptors or effect of ghrelin on human sperm [Ca²⁺]_i have not been investigated.

Functional significance of Ca²⁺-stores

The acrosome

AR is the fusion between the outer acrosomal membrane and the overlying plasma membrane. Fusion occurs at multiple points, resulting in vesiculation and loss of the fused outer acrosomal membrane/plasmalemma so that the acrosomal content is released and the inner acrosomal membrane becomes the new cell surface. Membrane fusion proteins from the SNARE family are present in the acrosomal region and may be integrated into microdomains that facilitate Ca²⁺-regulated membrane fusion in a manner that has been compared with events at presynaptic terminals (De Blas *et al.* 2005, Mayorga *et al.* 2007, Zittronski *et al.* 2010). Zona pellucida proteins interact with sperm surface receptors to activate a signalling cascade leading to AR (Florman *et al.* 2008) and release of acrosomal content at the surface of the zona may, in combination with hyper-activated motility, facilitate zona penetration. However, observation of mouse IVF using sperm with GFP-labelled acrosomes showed that, in addition to cells that undergo AR at the surface of the zona, sperm which arrive having already lost their acrosome (probably within the cumulus) may go on to penetrate the zona and fertilise (Jin *et al.* 2011). Physiological inducers of AR that have been studied (primarily mouse ZP3 and progesterone) induce Ca²⁺ influx across the plasma membrane and a sustained rise in [Ca²⁺]_i. O'Toole *et al.* (2000) provided pharmacological evidence that ZP3-induced AR in mouse sperm involved activation of store-operated Ca²⁺ influx downstream of Ca²⁺ store mobilisation. De Blas *et al.* (2002) showed that in streptolysin-permeabilised human sperm, mobilisation of the acrosomal Ca²⁺ store was a requirement for AR even when it was directly induced by introduction of Rab3A into the cytoplasm. Further studies using this permeabilised sperm model have provided information about the mechanisms by which fusion of the plasma and outer acrosomal membranes is regulated, resulting in a detailed model in which mobilisation of the acrosomal store is a central and necessary event (Ruete *et al.* 2014). Stimulation of PLC, leading to generation of IP₃ and activation of IP₃Rs in the outer acrosomal membrane may be key to this process (Fukami *et al.* 2001, 2003), but there is also evidence that the acrosomal membrane contains the NAADP-sensitive, Ca²⁺-permeable TPC (Calcraft *et al.* 2009) and that NAADP mobilises acrosomal Ca²⁺ in mouse sperm (Arndt *et al.* 2014).

The RNE and calreticulin-containing vesicles

A second area where Ca²⁺ storage organelles have been identified in mammalian sperm is at the sperm neck and midpiece (Fig. 1b). Mitochondria have mechanisms for accumulation and release of Ca²⁺ (Drago *et al.* 2011, Pizzo *et al.* 2012) and therefore may contribute to Ca²⁺

buffering and signalling in this part of the sperm. Inhibition of mitochondrial function in sea urchin sperm, using respiratory inhibitors or uncouplers, causes a rise in $[\text{Ca}^{2+}]_i$ and leads to activation of Ca^{2+} influx that has characteristics consistent with SOCs (Ardon *et al.* 2009). Treatment with mitochondrial uncouplers (2,4-dinitrophenol, carbonyl cyanide-4-(trifluoromethoxy)-phenyl-hydrazone) also increases $[\text{Ca}^{2+}]_i$ in human sperm (J Morris and S Publicover, unpublished observations). Mitochondria may thus contribute to shaping of Ca^{2+} signals in sperm. However, the primary stimulus-regulated Ca^{2+} storage in this part of the sperm is in the RNE and/or a second, apparently separate group of calreticulin-containing vesicular structures, both of which are sited at the sperm neck region and cytoplasmic droplet (Ho & Suarez 2001, 2003, Naaby-Hansen *et al.* 2001). Mobilisation of Ca^{2+} stored in these compartments regulates flagellar activity and treatment of mouse sperm with thimerosal stimulates hyperactivated motility by activating Ca^{2+} release from these organelles (Ho & Suarez 2001, Marquez *et al.* 2007). This effect occurs in the absence of extracellular Ca^{2+} and can be induced in sperm that are null for CatSper (Marquez *et al.* 2007). In mouse sperm the direction of the major, high-amplitude flagellar bend of hyperactivated sperm can be clearly characterised by reference to the hooked acrosomal cap (pro-hook or anti-hook). Sperm that became hyperactivated during capacitation *in vitro* (due to activation of CatSper) show pro-hook bends whereas those activated by store mobilisation (using thimerosal) show anti-hook bends (Chang & Suarez 2011). When sperm were observed interacting with the lining of isolated mouse oviducts, most hyperactivated cells showed anti-hook bending of the type that is elicited by store mobilisation (Chang & Suarez 2012).

In human sperm a similar effect of store mobilisation is observed. Thimerosal greatly increases the proportion of cells showing hyperactivated motility and 4-aminopyridine, which both alkalinises the cytoplasm (thus activating CatSper) and mobilises stored Ca^{2+} , is similarly potent (Alasmari *et al.* 2013a,b). In contrast, manipulations that should activate CatSper (elevation of pH_i , stimulation with progesterone or prostaglandin E_1) elevate $[\text{Ca}^{2+}]_i$ but have only minor stimulatory effects on the proportion of hyperactivated cells. Instead, these manipulations significantly increase penetration into viscous media (Alasmari *et al.* 2013a,b, Luo *et al.* 2014).

Model for interaction of CatSper channels and Ca^{2+} -stores

Patch clamp recordings have provided no evidence that conventional voltage-operated Ca^{2+} channels contribute to Ca^{2+} influx in mature mammalian sperm. In mouse sperm null for CatSper1 and the K^+ channel Slo3, only a small leak current was recorded even at high intracellular pH and strong depolarisation (Zeng *et al.*

2013). CatSper channels in mouse and human sperm are pH- and (weakly) voltage-sensitive, but in human sperm the channel is also ligand-sensitive. Established Ca^{2+} -mobilising agonists of human sperm such as progesterone and prostaglandin E_1 have been shown to activate CatSper but also a range of other small molecules including environmental pollutants such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (4,4'-DDT), *p,p'*-dichlorodiphenyldichloroethylene and 4-methylbenzylidene camphor are potent agonists (Tavares *et al.* 2013, Schiffer *et al.* 2014). In addition, agents used to demonstrate cyclic-nucleotide-activated Ca^{2+} influx (such as 8-Br-AMP) have been shown directly to activate CatSper by binding at the extracellular surface (Brenker *et al.* 2012). Thus it is possible that a significant proportion of the pharmacological data that apparently support the existence of multiple Ca^{2+} influx pathways in sperm are misleading and in fact reflect actions of the drugs on Ca^{2+} flux through CatSper channels (Brenker *et al.* 2012). Furthermore, experiments using CatSper null mice provide strong evidence that $[\text{Ca}^{2+}]_i$ elevation induced by solubilised ZP is dependent on Ca^{2+} influx through the CatSper channel in the flagellum, which then propagates to the head (Xia & Ren 2009; though see Cohen *et al.* (2014)). Interestingly, the ability of solubilised zona to induce AR was not diminished in CatSper-null sperm. These findings not only suggest that CatSper is the primary Ca^{2+} influx pathway in mammalian sperm, but also that in human sperm it may act as a Ca^{2+} -signalling 'hub' or 'node', such that the effects of diverse agonists are summated/integrated in the rate of Ca^{2+} influx into the flagellum (Brenker *et al.* 2012). This is an elegant and simple model for which there is already a significant body of data, but in its basic form it does not address the question of how a sperm can generate and use diverse $[\text{Ca}^{2+}]_i$ signals to control diverse Ca^{2+} -sensitive functions.

Mouse sperm null for CatSper are unable to hyperactivate (Carlson *et al.* 2003) and evidence from clinical cases suggests that CatSper is also required for normal levels of motility in human sperm (Avenarius *et al.* 2009, Smith *et al.* 2013). Why, then, is manipulation of Ca^{2+} stores more effective in inducing hyperactivated motility than treatments targeted to CatSper (Alasmari *et al.* 2013b)? We have proposed that CatSper activation acts as a trigger and consequent elevation of flagellar $[\text{Ca}^{2+}]_i$ stimulates secondary release of stored Ca^{2+} at the sperm neck, either by stimulating synthesis of IP_3 or by CICR, leading to hyperactivation (Alasmari *et al.* 2013b). Mathematical modelling of the Ca^{2+} signals induced by CatSper activation in mouse sperm suggests that diffusion of Ca^{2+} from the flagellum cannot explain the $[\text{Ca}^{2+}]_i$ increase that occurs at the sperm head upon activation of CatSper and that such a secondary Ca^{2+} release at the neck region must occur (Olson *et al.* 2010,

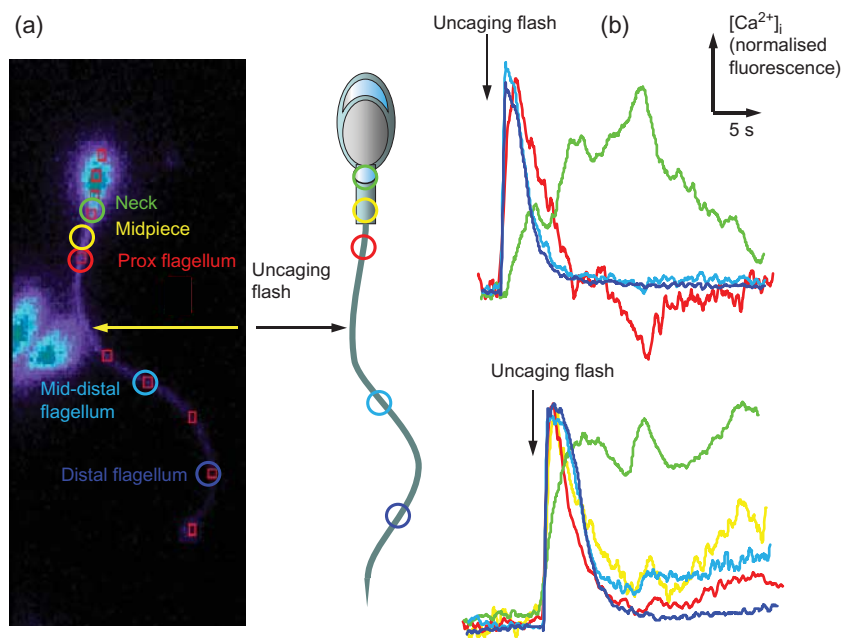


Figure 2 Ca^{2+} responses evoked in human sperm by uncaging of Ca^{2+} in the flagellum. Cells were labelled with fluo-4 and loaded with caged Ca^{2+} (NP-EGTA), then stimulated by an uncaging flash (360 nm laser) at the central flagellum (shown by arrow) while collecting images at 33 Hz. Changes in fluorescence, assessed at each of the positions shown by coloured circles in panel 'a', are plotted (normalised to minimum and maximum) in panel 'b' using the same colour code. Green, neck; yellow, midpiece; red, proximal flagellum; light blue, mid-distal flagellum; dark blue, distal flagellum.

2011, Li *et al.* 2014). Recently we have investigated the occurrence of such secondary responses in human sperm by uncaging Ca^{2+} in the principal piece of the flagellum. Uncaging induces a clear $[\text{Ca}^{2+}]_i$ transient in the flagellum that decays within 5–10 s. At the neck region of the sperm the transient is truncated and rises more slowly, consistent with diffusion of Ca^{2+} from the uncaged pool, but in a small proportion of cells (~10%) we have observed a late $[\text{Ca}^{2+}]_i$ response at the neck region, often including multiple peaks (Fig. 2). The low incidence of this secondary Ca^{2+} -mobilisation is consistent with our observation that, though direct release of stored Ca^{2+} can induce hyperactivated motility in the majority of human sperm, only a small proportion of cells hyperactivate upon activation of CatSper (Alasmari *et al.* 2013a,b).

Ca^{2+} -store-mediated $[\text{Ca}^{2+}]_i$ oscillations occur more readily in sperm incubated for a prolonged period (> 24 h) under capacitating conditions (Kirkman-Brown *et al.* 2004). Capacitation involves generation of ROS and RNS (Herrero *et al.* 1999, 2001, Aitken & Nixon 2013) and we have observed that store mobilisation is sensitised and induced by low concentrations of NO• donors, through a mechanism that involves protein S-nitrosylation (Machado-Oliveira *et al.* 2008). RyRs were detected in the human sperm nitrosoproteome (Lefievre *et al.* 2007) and it is well-established that IP_3 Rs and RyRs are sensitised by oxidative stress (Bootman *et al.* 1992, Sayers *et al.* 1993, Stoyanovsky *et al.* 1997, Meissner 2004, Bansaghi *et al.* 2014) (see ' Ca^{2+} stores and their regulation'). We propose that CICR from the sperm neck Ca^{2+} -store is regulated during capacitation, perhaps through the effects of oxidative stress on Ca^{2+} release channels (Alasmari *et al.* 2013b) (Fig. 3).

Final remarks

The central role of $[\text{Ca}^{2+}]_i$ signalling in the physiology of mammalian sperm and the pivotal importance of CatSper in this process are well established – mice null for CatSper are infertile (Ren *et al.* 2001) and in men CatSper lesions are associated with impaired sperm

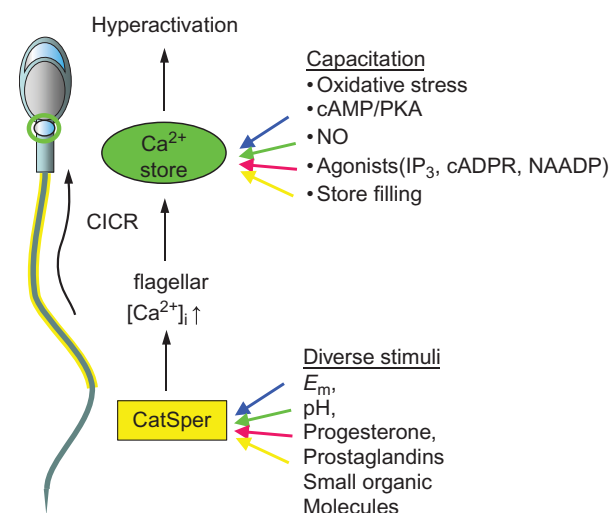


Figure 3 Model for triggering/regulation of CatSper-activated hyperactivation. CatSper channels in the flagellum (yellow box; shown by yellow shading on sperm flagellum) are activated by diverse stimuli including intracellular pH (pH_i), membrane potential (E_m), progesterone, prostaglandins and other organic molecules. Ca^{2+} from the flagellum diffuses forward, raising $[\text{Ca}^{2+}]_i$ at the sperm neck and can mobilise stored Ca^{2+} by Ca^{2+} -induced Ca^{2+} release (CICR). Susceptibility of the store to CICR is potentially regulated/sensitised by processes occurring during capacitation, including cAMP signalling, oxidative stress and S-nitrosylation as well as Ca^{2+} store filling and effects of agonists on Ca^{2+} -store release channels.

function (Avidan *et al.* 2003, Avenarius *et al.* 2009, Zhang *et al.* 2009, Smith *et al.* 2013). The available evidence suggests that Ca^{2+} -stores also play important roles in both AR and the regulation of motility. Future studies should address the mechanisms by which store mobilisation is achieved (both by CICR and by agonist-induced generation of Ca^{2+} -mobilising 2nd messengers) and regulated, particularly the significance of capacitation in Ca^{2+} -store filling and in sensitising Ca^{2+} release mechanisms. Also, similarly to the important species differences in expression and function of sperm ion channels between human and mouse sperm (Brenker *et al.* 2014, Miller *et al.* 2015), there may be differences in store-regulation and/or function between species. An intriguing possibility is that, at least in human sperm, it may prove possible to bypass the effects on motility of lesions in the expression, function or regulation of CatSper channels by pharmacological activation of stored Ca^{2+} release.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

J Correia was supported by the Wellcome Trust (grant number 086470).

References

- Adachi T, Weisbrod RM, Pimentel DR, Ying J, Sharov VS, Schoneich C & Cohen RA 2004 S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. *Nature Medicine* **10** 1200–1207. (doi:10.1038/nm1119)
- Aitken RJ & Nixon B 2013 Sperm capacitation: a distant landscape glimpsed but unexplored. *Molecular Human Reproduction* **19** 785–793. (doi:10.1093/molehr/gat067)
- Alasmari W, Barratt C, Publicover S, Whalley K, Foster E, Kay V, Silva SMD & Oxenham S 2013a The incidence and clinical significance of defects in calcium signalling pathways mediating human sperm hyperactivation in donors and sub fertile patients. *Human Reproduction* **28** 866–876. (doi:10.1093/humrep/des467)
- Alasmari W, Costello S, Correia J, Oxenham SK, Morris J, Fernandes L, Ramalho-Santos J, Kirkman-Brown J, Michelangeli F, Publicover S *et al.* 2013b Ca^{2+} signals generated by CatSper and Ca^{2+} regulate different behaviours in human sperm. *Journal of Biological Chemistry* **288** 6248–6258. (doi:10.1074/jbc.M112.439356)
- Aquila S, Guido C, Middea E, Perrotta I, Bruno R, Pellegrino M & Ando S 2009 Human male gamete endocrinology: $1\alpha, 25\text{-dihydroxyvitamin D}_3$ ($1,25(\text{OH})_2\text{D}_3$) regulates different aspects of human sperm biology and metabolism. *Reproductive Biology and Endocrinology* **7** 140. (doi:10.1186/1477-7827-7-140)
- Ardon F, Rodriguez-Miranda E, Beltran C, Hernandez-Cruz A & Darszon A 2009 Mitochondrial inhibitors activate influx of external Ca^{2+} in sea urchin sperm. *Biochimica et Biophysica Acta* **1787** 15–24. (doi:10.1016/j.bbabi.2008.10.003)
- Arndt L, Castonguay J, Arlt E, Meyer D, Hassan S, Borth H, Zierler S, Wennemuth G, Breit A, Biel M *et al.* 2014 NAADP and the two-pore channel protein 1 participate in the acrosome reaction in mammalian spermatozoa. *Molecular Biology of the Cell* **25** 948–964. (doi:10.1091/mbc.E13-09-0523)
- Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, Najmabadi H & Smith RJ 2009 Human male infertility caused by mutations in the CATSPER1 channel protein. *American Journal of Human Genetics* **84** 505–510. (doi:10.1016/j.ajhg.2009.03.004)
- Avidan N, Tamary H, Dgany O, Cattani D, Pariente A, Thulliez M, Borot N, Moati L, Barthelme A, Shalmon L *et al.* 2003 CATSPER2, a human autosomal nonsyndromic male infertility gene. *European Journal of Human Genetics* **11** 497–502. (doi:10.1038/sj.ejhg.5200991)
- Bansaghi S, Golenar T, Madesh M, Csordas G, Ramachandra Rao S, Sharma K, Yule DI, Joseph SK & Hajnoczky G 2014 Isoform- and species-specific control of inositol 1,4,5-trisphosphate (IP₃) receptors by reactive oxygen species. *Journal of Biological Chemistry* **289** 8170–8181. (doi:10.1074/jbc.M113.504159)
- Beltramo M, Dardente H, Cayla X & Caraty A 2014 Cellular mechanisms and integrative timing of neuroendocrine control of GnRH secretion by kisspeptin. *Molecular and Cellular Endocrinology* **382** 387–399. (doi:10.1016/j.mce.2013.10.015)
- Berridge MJ, Bootman MD & Roderick HL 2003 Calcium signalling: dynamics, homeostasis and remodelling. *Nature Reviews. Molecular and Cellular Biology* **4** 517–529. (doi:10.1038/nrm1155)
- Bezprozvanny I, Watras J & Ehrlich BE 1991 Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351** 751–754. (doi:10.1038/351751a0)
- Billington RA, Ho A & Genazzani AA 2002 Nicotinic acid adenine dinucleotide phosphate (NAADP) is present at micromolar concentrations in sea urchin spermatozoa. *Journal of Physiology* **544** 107–112. (doi:10.1113/jphysiol.2002.030098)
- Billington RA, Harper C, Bellomo EA, Publicover S, Barratt CL & Genazzani AA 2006 Characterization of cyclic adenine dinucleotide phosphate ribose levels in human spermatozoa. *Fertility and Sterility* **86** 891–898. (doi:10.1016/j.fertnstert.2006.03.030)
- Blackmore PF 1993 Thapsigargin elevates and potentiates the ability of progesterone to increase intracellular free calcium in human sperm: possible role of perinuclear calcium. *Cell Calcium* **14** 53–60. (doi:10.1016/0143-4160(93)90018-2)
- Blomberg Jensen M 2014 Vitamin D and male reproduction. *Nature Reviews. Endocrinology* **10** 175–186. (doi:10.1038/nrendo.2013.262)
- Blomberg Jensen M, Nielsen JE, Jorgensen A, Rajpert-De Meyts E, Kristensen DM, Jorgensen N, Skakkebaek NE, Juul A & Leffers H 2010 Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Human Reproduction* **25** 1303–1311. (doi:10.1093/humrep/deq024)
- Blomberg Jensen M, Bjerrum PJ, Jessen TE, Nielsen JE, Joensen UN, Olesen IA, Petersen JH, Juul A, Dissing S & Jorgensen N 2011 Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Human Reproduction* **26** 1307–1317. (doi:10.1093/humrep/der059)
- Blomberg Jensen M, Jorgensen A, Nielsen JE, Bjerrum PJ, Skalkam M, Petersen JH, Egeberg DL, Bangsbo S, Andersen AN, Skakkebaek NE *et al.* 2012 Expression of the vitamin D metabolizing enzyme CYP24A1 at the annulus of human spermatozoa may serve as a novel marker of semen quality. *International Journal of Andrology* **35** 499–510. (doi:10.1111/j.1365-2605.2012.01256.x)
- Bootman MD, Taylor CW & Berridge MJ 1992 The thiol reagent, thimerosal, evokes Ca^{2+} spikes in HeLa cells by sensitizing the inositol 1,4,5-trisphosphate receptor. *Journal of Biological Chemistry* **267** 25113–25119.
- Brenker C, Goodwin N, Weyand I, Kashikar ND, Naruse M, Krahling M, Muller A, Kaupp UB & Strunker T 2012 The Ca^{2+} -activated K^{+} current of human sperm is mediated by Slo3. *EMBO Journal* **31** 1654–1665. (doi:10.1038/emboj.2012.30)
- Brenker C, Zhou Y, Muller A, Echeverry F, Trotschel C, Poetsch A, Xia X, Bonigk W, Lingle C, Kaupp U *et al.* 2014 Slo3 in human sperm – a K^{+} channel activated by Ca^{2+} . *eLife* **3** e01438. (doi:10.7554/eLife.01438)
- Cahalan MD 2009 STIMulating store-operated Ca^{2+} entry. *Nature Cell Biology* **11** 669–677. (doi:10.1038/ncb0609-669)

- Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang KT *et al.* 2009 NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature* **459** 596–600. (doi:10.1038/nature08030)
- Camina JP, Carreira MC, Micic D, Pombo M, Kelestimur F, Dieguez C & Casanueva FF 2003 Regulation of ghrelin secretion and action. *Endocrine* **22** 5–12. (doi:10.1385/ENDO:22:1:5)
- Camors E & Valdivia HH 2014 CaMKII regulation of cardiac ryanodine receptors and inositol triphosphate receptors. *Frontiers in Pharmacology* **5** 101. (doi:10.3389/fphar.2014.00101)
- Cancela JM, Churchill GC & Galione A 1999 Coordination of agonist-induced Ca^{2+} -signalling patterns by NAADP in pancreatic acinar cells. *Nature* **399** 74–77. (doi:10.1038/18032)
- Cang C, Zhou Y, Navarro B, Seo YJ, Aranda K, Shi L, Battaglia-Hsu S, Nissim I, Clapham DE & Ren D 2013 mTOR regulates lysosomal ATP-sensitive two-pore Na^{+} channels to adapt to metabolic state. *Cell* **152** 778–790. (doi:10.1016/j.cell.2013.01.023)
- Carlson AE, Westenbroek RE, Quill T, Ren D, Clapham DE, Hille B, Garbers DL & Babcock DF 2003 CatSper1 required for evoked Ca^{2+} entry and control of flagellar function in sperm. *PNAS* **100** 14864–14868. (doi:10.1073/pnas.2536658100)
- Castellano LE, Trevino CL, Rodriguez D, Serrano CJ, Pacheco J, Tsutsumi V, Felix R & Darszon A 2003 Transient receptor potential (TRPC) channels in human sperm: expression, cellular localization and involvement in the regulation of flagellar motility. *FEBS Letters* **541** 69–74. (doi:10.1016/S0014-5793(03)00305-3)
- Chandrasekran A, Isa I, Dudhia J, Thrasher AJ, Dibb N, Casimir C, Readhead C & Winston R 2014a Lentiviral vector transduction of spermatozoa as a tool for the study of early development. *FEBS Open Bio* **4** 266–275. (doi:10.1016/j.fob.2014.02.008)
- Chandrasekran A, Sarkar R, Thrasher A, Fraser SE, Dibb N, Casimir C, Winston R & Readhead C 2014b Efficient generation of transgenic mice by lentivirus-mediated modification of spermatozoa. *FASEB Journal* **28** 569–576. (doi:10.1096/fj.13-233999)
- Chang H & Suarez SS 2011 Two distinct Ca^{2+} signaling pathways modulate sperm flagellar beating patterns in mice. *Biological Reproduction* **85** 296–305. (doi:10.1095/biolreprod.110.089789)
- Chang H & Suarez SS 2012 Unexpected flagellar movement patterns and epithelial binding behavior of mouse sperm in the oviduct. *Biological Reproduction* **86** 141–148. (doi:10.1095/biolreprod.111.096578)
- Chini EN, Thompson MA, Chini CC & Dousa TP 1997 Cyclic ADP-ribose signaling in sea urchin gametes: metabolism in spermatozoa. *American Journal of Physiology* **272** C416–C420.
- Churchill GC, Okada Y, Thomas JM, Genazzani AA, Patel S & Galione A 2002 NAADP mobilizes Ca^{2+} from reserve granules, lysosome-related organelles, in sea urchin eggs. *Cell* **111** 703–708. (doi:10.1016/S0092-8674(02)01082-6)
- Cohen R, Buttke DE, Asano A, Mukai C, Nelson JL, Ren D, Miller RJ, Cohen-Kutner M, Atlas D & Travis AJ 2014 Lipid modulation of calcium flux through $\text{CaV}2.3$ regulates acrosome exocytosis and fertilization. *Developmental Cell* **28** 310–321. (doi:10.1016/j.devcel.2014.01.005)
- Colyer J 1998 Phosphorylation states of phospholamban. *Annals of New York Academy of Sciences* **853** 79–91. (doi:10.1111/j.1749-6632.1998.tb08258.x)
- Cosker F, Cheviron N, Yamasaki M, Menteyne A, Lund FE, Moutin MJ, Galione A & Cancela JM 2010 The ecto-enzyme CD38 is a mammalian NAADP synthase which couples receptor activation to Ca^{2+} mobilization from lysosomes. *Journal of Biological Chemistry* **285** 38251–38259. (doi:10.1074/jbc.M110.125864)
- Costello S, Michelangeli F, Nash K, Lefievre L, Morris J, Machado-Oliveira G, Barratt C, Kirkman-Brown J & Publicover S 2009 Ca^{2+} stores in sperm: their identities and functions. *Reproduction* **138** 425–437. (doi:10.1530/REP-09-0134)
- Csordas G & Hajnoczky G 2009 SR/ER-mitochondrial local communication: calcium and ROS. *Biochimica et Biophysica Acta* **1787** 1352–1362. (doi:10.1016/j.bbabi.2009.06.004)
- Darszon A, Trevino CL, Wood C, Galindo B, Rodriguez-Miranda E, Acevedo JJ, Hernandez-Gonzalez EO, Beltran C, Martinez-Lopez P & Nishigaki T 2007 Ion channels in sperm motility and capacitation. *Society of Reproduction and Fertility Supplement* **65** 229–244.
- Darszon A, Nishigaki T, Beltran C & Trevino CL 2011 Calcium channels in the development, maturation, and function of spermatozoa. *Physiological Reviews* **91** 1305–1355. (doi:10.1152/physrev.00028.2010)
- Darszon A, Sanchez-Cardenas C, Orta G, Sanchez-Tusie AA, Beltran C, Lopez-Gonzalez I, Granados-Gonzalez G & Trevino CL 2012 Are TRP channels involved in sperm development and function? *Cell and Tissue Research* **349** 749–764. (doi:10.1007/s00441-012-1397-5)
- De Blas G, Michaut M, Trevino CL, Tomes CN, Yunes R, Darszon A & Mayorga LS 2002 The intraacrosomal calcium pool plays a direct role in acrosomal exocytosis. *Journal of Biological Chemistry* **277** 49326–49331. (doi:10.1074/jbc.M208587200)
- De Blas GA, Roggero CM, Tomes CN & Mayorga LS 2005 Dynamics of SNARE assembly and disassembly during sperm acrosomal exocytosis. *PLoS Biology* **3** e323. (doi:10.1371/journal.pbio.0030323)
- Dragileva E, Rubinstein S & Breitbart H 1999 Intracellular Ca^{2+} - Mg^{2+} -ATPase regulates calcium influx and acrosomal exocytosis in bull and ram spermatozoa. *Biological Reproduction* **61** 1226–1234. (doi:10.1095/biolreprod61.5.1226)
- Drago I, Pizzo P & Pozzan T 2011 After half a century mitochondrial calcium in- and efflux machineries reveal themselves. *EMBO Journal* **30** 4119–4125. (doi:10.1038/emboj.2011.337)
- Dyer JL, Mobasher H, Lea EJ, Dawson AP & Michelangeli F 2003 Differential effect of PKA on the Ca^{2+} release kinetics of the type I and III InsP_3 receptors. *Biochemistry and Biophysics Research Communications* **302** 121–126. (doi:10.1016/S0006-291X(03)00120-7)
- Espino J, Mediero M, Lozano GM, Bejarano I, Ortiz A, Garcia JF, Pariente JA & Rodriguez AB 2009 Reduced levels of intracellular calcium releasing in spermatozoa from asthenozoospermic patients. *Reproductive Biology and Endocrinology* **7** 11. (doi:10.1186/1477-7827-7-11)
- Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, Wang Y, Muend S, Kenny PA, Sukumar S, Roberts-Thomson SJ *et al.* 2010 Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell* **143** 84–98. (doi:10.1016/j.cell.2010.08.040)
- Florman HM, Jungnickel MK & Sutton KA 2008 Regulating the acrosome reaction. *International Journal of Developmental Biology* **52** 503–510. (doi:10.1387/ijdb.082696hf)
- Fukami K, Nakao K, Inoue T, Kataoka Y, Kurokawa M, Fissore RA, Nakamura K, Katsuki M, Mikoshiba K, Yoshida N *et al.* 2001 Requirement of phospholipase Cdelta4 for the zona pellucida-induced acrosome reaction. *Science* **292** 920–923. (doi:10.1126/science.1059042)
- Fukami K, Yoshida M, Inoue T, Kurokawa M, Fissore RA, Yoshida N, Mikoshiba K & Takenawa T 2003 Phospholipase Cdelta4 is required for Ca^{2+} mobilization essential for acrosome reaction in sperm. *Journal of Cell Biology* **161** 79–88. (doi:10.1083/jcb.200210057)
- Genazzani AA, Mezna M, Summerhill RJ, Galione A & Michelangeli F 1997 Kinetic properties of nicotinic acid adenine dinucleotide phosphate-induced Ca^{2+} release. *Journal of Biological Chemistry* **272** 7669–7675. (doi:10.1074/jbc.272.12.7669)
- Gur Y & Breitbart H 2008 Protein synthesis in sperm: dialog between mitochondria and cytoplasm. *Molecular and Cellular Endocrinology* **282** 45–55. (doi:10.1016/j.mce.2007.11.015)
- Guse AH 2015 Calcium mobilizing second messengers derived from NAD. *Biochimica et Biophysica Acta*. In press. (doi:10.1016/j.bbapap.2014.12.015)
- Hamilton SL & Reid MB 2000 RyR1 modulation by oxidation and calmodulin. *Antioxidants & Redox Signaling* **2** 41–45. (doi:10.1089/ars.2000.2.1-41)
- Harper CV, Barratt CL & Publicover SJ 2004 Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of $[\text{Ca}^{2+}]_i$ oscillations and cyclical transitions in flagellar beating. *Journal of Biological Chemistry* **279** 46315–46325. (doi:10.1074/jbc.M401194200)
- Harper C, Wootton L, Michelangeli F, Lefievre L, Barratt C & Publicover S 2005 Secretory pathway Ca^{2+} -ATPase (SPCA1) Ca^{2+} pumps, not SERCAs, regulate complex $[\text{Ca}^{2+}]_i$ signals in human spermatozoa. *Journal of Cell Science* **118** 1673–1685. (doi:10.1242/jcs.02297)
- Herrero MB, de Lamirande E & Gagnon C 1999 Nitric oxide regulates human sperm capacitation and protein-tyrosine phosphorylation *in vitro*. *Biological Reproduction* **61** 575–581. (doi:10.1095/biolreprod61.3.575)

- Herrero MB, de Lamirande E & Gagnon C 2001 Tyrosine nitration in human spermatozoa: a physiological function of peroxynitrite, the reaction product of nitric oxide and superoxide. *Molecular Human Reproduction* **7** 913–921. (doi:10.1093/molehr/7.10.913)
- Ho HC & Suarez SS 2001 An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca^{2+} store is involved in regulating sperm hyperactivated motility. *Biological Reproduction* **65** 1606–1615. (doi:10.1095/biolreprod65.5.1606)
- Ho HC & Suarez SS 2003 Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. *Biological Reproduction* **68** 1590–1596. (doi:10.1095/biolreprod.102.011320)
- Hsu MC, Wang JY, Lee YJ, Jong DS, Tsui KH & Chiu CH 2014 Kisspeptin modulates fertilization capacity of mouse spermatozoa. *Reproduction* **147** 835–845. (doi:10.1530/REP-13-0368)
- Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K & Hirohashi N 2011 Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during *in vitro* fertilization. *PNAS* **108** 4892–4896. (doi:10.1073/pnas.1018202108)
- Kirkman-Brown JC, Barratt CL & Publicover SJ 2004 Slow calcium oscillations in human spermatozoa. *Biochemistry Journal* **378** 827–832. (doi:10.1042/BJ20031368)
- Kuroda Y, Kaneko S, Yoshimura Y, Nozawa S & Mikoshiba K 1999 Are there inositol 1,4,5-trisphosphate (IP₃) receptors in human sperm? *Life Sciences* **65** 135–143. (doi:10.1016/S0024-3205(99)00230-1)
- Lawson C, Dorval V, Goupil S & Leclerc P 2007 Identification and localisation of SERCA 2 isoforms in mammalian sperm. *Molecular Human Reproduction* **13** 307–316. (doi:10.1093/molehr/gam012)
- Lefevre L, Chen Y, Conner SJ, Scott JL, Publicover SJ, Ford WC & Barratt CL 2007 Human spermatozoa contain multiple targets for protein S-nitrosylation: an alternative mechanism of the modulation of sperm function by nitric oxide? *Proteomics* **7** 3066–3084. (doi:10.1002/pmic.200700254)
- Lefevre L, Nash K, Mansell S, Costello S, Punt E, Correia J, Morris J, Kirkman-Brown J, Wilson SM, Barratt CL *et al.* 2012 2-APB-potentiated channels amplify CatSper-induced Ca^{2+} signals in human sperm. *Biochemistry Journal* **448** 189–200. (doi:10.1042/BJ20120339)
- Li LF, Xiang C, Zhu YB & Qin KR 2014 Modeling of progesterone-induced intracellular calcium signaling in human spermatozoa. *Journal of Theoretical Biology* **351** 58–66. (doi:10.1016/j.jtbi.2014.02.026)
- Lin-Moshier Y, Walseth EF, Churamani D, Davidson SM, Slama JT, Hooper R, Brailoiu E, Patel S & Marchant JS 2012 Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells. *Journal of Biological Chemistry* **287** 2296–2307. (doi:10.1074/jbc.M111.305813)
- Lishko PV, Botchkina IL & Kirichok Y 2011 Progesterone activates the principal Ca^{2+} channel of human sperm. *Nature* **471** 387–391. (doi:10.1038/nature09767)
- Liu X, Lee K & Herbison AE 2008 Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. *Endocrinology* **149** 4605–4614. (doi:10.1210/en.2008-0321)
- Luik RM, Wu MM, Buchanan J & Lewis RS 2006 The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *Journal of Cell Biology* **174** 815–825. (doi:10.1083/jcb.200604015)
- Lukaszyk A, Kotwicka M, Jankowska A, Kasprzak A, Rucinski M, Sterzynska K, Ziolkowska A, Sawinski P & Ruchala M 2012 Expression of ghrelin receptor (GHSR-1a) in rat epididymal spermatozoa and the effects of its activation. *Reproductive Biology* **12** 293–300. (doi:10.1016/j.repbio.2012.09.002)
- Luo T, Li N, He YQ, Weng SQ, Wang T, Zou QX & Zeng XH 2014 Emodin inhibits human sperm functions by reducing sperm [Ca] and tyrosine phosphorylation. *Reproductive Toxicology* **51C** 14–21. (doi:10.1016/j.reprotox.2014.11.007) Epub 2014 Nov 22.
- Machado-Oliveira G, Lefevre L, Ford C, Herrero MB, Barratt C, Connolly TJ, Nash K, Morales-Garcia A, Kirkman-Brown J & Publicover S 2008 Mobilisation of Ca^{2+} stores and flagellar regulation in human sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. *Development* **135** 3677–3686. (doi:10.1242/dev.024521)
- Marquez B, Ignatz G & Suarez SS 2007 Contributions of extracellular and intracellular Ca^{2+} to regulation of sperm motility: release of intracellular stores can hyperactivate CatSper1 and CatSper2 null sperm. *Developmental Biology* **303** 214–221. (doi:10.1016/j.ydbio.2006.11.007)
- Mayorga LS, Tomes CN & Belmonte SA 2007 Acrosomal exocytosis, a special type of regulated secretion. *IUBMB Life* **59** 286–292. (doi:10.1080/15216540701222872)
- Meissner G 2004 Molecular regulation of cardiac ryanodine receptor ion channel. *Cell Calcium* **35** 621–628. (doi:10.1016/j.ceca.2004.01.015)
- Menteyne A, Burdakov A, Charpentier G, Petersen OH & Cancela JM 2006 Generation of specific Ca^{2+} signals from Ca^{2+} stores and endocytosis by differential coupling to messengers. *Current Biology* **16** 1931–1937. (doi:10.1016/j.cub.2006.07.070)
- Michelangeli F & East JM 2011 A diversity of SERCA Ca^{2+} pump inhibitors. *Biochemical Society Transactions* **39** 789–797. (doi:10.1042/BST0390789)
- Michelangeli F, Ogunbayo OA & Wootton LL 2005 A plethora of interacting organellar Ca^{2+} stores. *Current Opinion in Cell Biology* **17** 135–140. (doi:10.1016/j.ceb.2005.01.005)
- Miller D & Ostermeier GC 2006 Spermatozoal RNA: why is it there and what does it do? *Gynecologic and Obstetric Investigation* **34** 840–846.
- Miller D, Ostermeier GC & Krawetz SA 2005 The controversy, potential and roles of spermatozoal RNA. *Trends in Molecular Medicine* **11** 156–163. (doi:10.1016/j.molmed.2005.02.006)
- Miller MR, Mansell SA, Meyers SA & Lishko PV 2015 Flagellar ion channels of sperm: similarities and differences between species. *Cell Calcium*. In press. (doi:10.1016/j.ceca.2014.10.009)
- Missiaen L, Taylor CW & Berridge MJ 1991 Spontaneous calcium release from inositol trisphosphate-sensitive calcium stores. *Nature* **352** 241–244. (doi:10.1038/352241a0)
- Moretti E, Vindigni C, Tripodi SA, Mazzi L, Nuti R, Figura N & Collodel G 2014 Immunolocalisation of ghrelin and obestatin in human testis, seminal vesicles, prostate and spermatozoa. *Andrologia* **46** 979–985. (doi:10.1111/and.12183)
- Morgan AJ & Galione A 2014 Two-pore channels (TPCs): current controversies. *BioEssays* **36** 173–183. (doi:10.1002/bies.201300118)
- Morris J, Jones S, Howl J, Lukanowska M, Lefevre L & Publicover S 2015 Cell penetrating peptides, targeting the regulation of store-operated channels, slow decay of the progesterone-induced $[\text{Ca}^{2+}]_i$ signal in human sperm. *Molecular Human Reproduction*. In press. (doi:10.1093/molehr/gav019)
- Naaby-Hansen S, Wolkowicz MJ, Klotz K, Bush LA, Westbrook VA, Shibahara H, Shetty J, Conrod SA, Reddi PP, Shannon J *et al.* 2001 Colocalization of the inositol 1,4,5-trisphosphate receptor and calreticulin in the equatorial segment and in membrane bounded vesicles in the cytoplasmic droplet of human spermatozoa. *Molecular Human Reproduction* **7** 923–933. (doi:10.1093/molehr/7.10.923)
- Navarro-Borelly L, Somasundaram A, Yamashita M, Ren D, Miller RJ & Prakriya M 2008 STIM1–Orai1 interactions and Orai1 conformational changes revealed by live-cell FRET microscopy. *Journal of Physiology* **586** 5383–5401. (doi:10.1113/jphysiol.2008.162503)
- Ogunbayo OA, Zhu Y, Rossi D, Sorrentino V, Ma J, Zhu MX & Evans AM 2011 Cyclic adenosine diphosphate ribose activates ryanodine receptors, whereas NAADP activates two-pore domain channels. *Journal of Biological Chemistry* **286** 9136–9140. (doi:10.1074/jbc.M110.202002)
- Olson SD, Suarez SS & Fauci LJ 2010 A model of CatSper channel mediated calcium dynamics in mammalian spermatozoa. *Bulletin of Mathematical Biology* **72** 1925–1946. (doi:10.1007/s11538-010-9516-5)
- Olson SD, Fauci LJ & Suarez SS 2011 Mathematical modeling of calcium signaling during sperm hyperactivation. *Molecular Human Reproduction* **17** 500–510. (doi:10.1093/molehr/gar040)
- O'Toole CM, Arnoult C, Darszon A, Steinhardt RA & Florman HM 2000 Ca^{2+} entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. *Molecular Biology of the Cell* **11** 1571–1584. (doi:10.1091/mbc.11.5.1571)
- Park KH, Kim BJ, Kang J, Nam TS, Lim JM, Kim HT, Park JK, Kim YG, Chae SW & Kim UH 2011 Ca^{2+} signaling tools acquired from prostasomes are required for progesterone-induced sperm motility. *Science Signaling* **4** ra31. (doi:10.1126/scisignal.2001595)

- Peiter E, Maathuis FJ, Mills LN, Knight H, Pelloux J, Hetherington AM & Sanders D 2005 The vacuolar Ca^{2+} -activated channel TPC1 regulates germination and stomatal movement. *Nature* **434** 404–408. (doi:10.1038/nature03381)
- Pielecka-Fortuna J, Chu Z & Moenter SM 2008 Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. *Endocrinology* **149** 1979–1986. (doi:10.1210/en.2007-1365)
- Pinto FM, Cejudo-Roman A, Ravina CG, Fernandez-Sanchez M, Martin-Lozano D, Illanes M, Tena-Sempere M & Candenas ML 2012 Characterization of the kisspeptin system in human spermatozoa. *International Journal of Andrology* **35** 63–73. (doi:10.1111/j.1365-2605.2011.01177.x)
- Pizzo P, Drago I, Filadi R & Pozzan T 2012 Mitochondrial Ca^{2+} homeostasis: mechanism, role, and tissue specificities. *Pflügers Archiv* **464** 3–17. (doi:10.1007/s00424-012-1122-y)
- Publicover S, Harper CV & Barratt C 2007 $[\text{Ca}^{2+}]_i$ signalling in sperm-making the most of what you've got. *Nature Cell Biology* **9** 235–242. (doi:10.1038/ncb0307-235)
- Ren D & Xia J 2010 Calcium signaling through CatSper channels in mammalian fertilization. *Physiology* **25** 165–175. (doi:10.1152/physiol.00049.2009)
- Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL & Clapham DE 2001 A sperm ion channel required for sperm motility and male fertility. *Nature* **413** 603–609. (doi:10.1038/35098027)
- Ruas M, Davis LC, Chen CC, Morgan AJ, Chuang KT, Walseth TF, Grimm C, Garnham C, Powell T, Platt N *et al.* 2015 Expression of Ca^{2+} -permeable two-pore channels rescues NAADP signalling in TPC-deficient cells. *EMBO Journal*. In press. (10.15252/embj.201490009)
- Ruete MC, Lucchesi O, Bustos MA & Tomes CN 2014 Epac, Rap and Rab3 act in concert to mobilize calcium from sperm's acrosome during exocytosis. *Cell Communication and Signaling* **12** 43. (doi:10.1186/s12964-014-0043-0)
- Sanchez-Tusie AA, Vasudevan SR, Churchill GC, Nishigaki T & Trevino CL 2014 Characterization of NAADP-mediated calcium signaling in human spermatozoa. *Biochemistry and Biophysics Research Communications* **443** 531–536. (doi:10.1016/j.bbrc.2013.12.011)
- Sayers LG, Brown GR, Michell RH & Michelangeli F 1993 The effects of thimerosal on calcium uptake and inositol 1,4,5-trisphosphate-induced calcium release in cerebellar microsomes. *Biochemistry Journal* **289** (Pt 3) 883–887.
- Schiffer C, Muller A, Egeberg DL, Alvarez L, Brenker C, Rehfeld A, Frederiksen H, Waschle B, Kaupp UB, Balbach M *et al.* 2014 Direct action of endocrine disrupting chemicals on human sperm. *EMBO Reports* **15** 758–765. (doi:10.15252/embr.201438869)
- Seo MD, Velamakanni S, Ishiyama N, Stathopoulos PB, Rossi AM, Khan SA, Dale P, Li C, Ames JB, Ikura M *et al.* 2012 Structural and functional conservation of key domains in InsP3 and ryanodine receptors. *Nature* **483** 108–112. (doi:10.1038/nature10751)
- Sharov VS, Dremina ES, Galeva NA, Williams TD & Schoneich C 2006 Quantitative mapping of oxidation-sensitive cysteine residues in SERCA *in vivo* and *in vitro* by HPLC-electrospray-tandem MS: selective protein oxidation during biological aging. *Biochemistry Journal* **394** 605–615. (doi:10.1042/BJ20051214)
- Smith JF, Syritsyna O, Fellous M, Serres C, Mannowetz N, Kirichok Y & Lishko PV 2013 Disruption of the principal, progesterone-activated sperm Ca^{2+} channel in a CatSper2-deficient infertile patient. *PNAS* **110** 6323–6328. (doi:10.1073/pnas.1216588110)
- Stoyanovsky D, Murphy T, Anno PR, Kim YM & Salama G 1997 Nitric oxide activates skeletal and cardiac ryanodine receptors. *Cell Calcium* **21** 19–29. (doi:10.1016/S0143-4160(97)90093-2)
- Strunker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R & Kaupp UB 2011 The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm. *Nature* **471** 382–386. (doi:10.1038/nature09769)
- Tavares RS, Mansell S, Barratt CL, Wilson SM, Publicover SJ & Ramalho-Santos J 2013 p,p' -DDE activates CatSper and compromises human sperm function at environmentally relevant concentrations. *Human Reproduction* **28** 3167–3177. (doi:10.1093/humrep/det372)
- Thomas P & Meizel S 1989 Phosphatidylinositol 4,5-bisphosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon Ca^{2+} influx. *Biochemistry Journal* **264** 539–546.
- Trevino CL, Santi CM, Beltran C, Hernandez-Cruz A, Darszon A & Lomeli H 1998 Localisation of inositol trisphosphate and ryanodine receptors during mouse spermatogenesis: possible functional implications. *Zygote* **6** 159–172. (doi:10.1017/S0967199498000094)
- Vanoevelen J, Dode L, Van Baelen K, Fairclough RJ, Missiaen L, Raeymaekers L & Wuytack F 2005 The secretory pathway $\text{Ca}^{2+}/\text{Mn}^{2+}$ -ATPase 2 is a Golgi-localized pump with high affinity for Ca^{2+} ions. *Journal of Biological Chemistry* **280** 22800–22808. (doi:10.1074/jbc.M501026200)
- Vasudevan SR, Galione A & Churchill GC 2008 Sperm express a Ca^{2+} -regulated NAADP synthase. *Biochemistry Journal* **411** 63–70. (doi:10.1042/BJ20071616)
- Wang Y, Deng X, Zhou Y, Hendron E, Mancarella S, Ritchie MF, Tang XD, Baba Y, Kurosaki T, Mori Y *et al.* 2009 STIM protein coupling in the activation of Orai channels. *PNAS* **106** 7391–7396. (doi:10.1073/pnas.0900293106)
- Wang X, Zhang X, Dong XP, Samie M, Li X, Cheng X, Goschka A, Shen D, Zhou Y, Harlow J *et al.* 2012 TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. *Cell* **151** 372–383. (doi:10.1016/j.cell.2012.08.036)
- Wootton LL, Argent CC, Wheatley M & Michelangeli F 2004 The expression, activity and localisation of the secretory pathway Ca^{2+} -ATPase (SPCA1) in different mammalian tissues. *Biochimica et Biophysica Acta* **1664** 189–197. (doi:10.1016/j.bbame.2004.05.009)
- Wu MM, Buchanan J, Luik RM & Lewis RS 2006 Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *Journal of Cell Biology* **174** 803–813. (doi:10.1083/jcb.200604014)
- Xia J & Ren D 2009 Egg coat proteins activate calcium entry into mouse sperm via CATSPER channels. *Biological Reproduction* **80** 1092–1098. (doi:10.1095/biolreprod.108.074039)
- Yamashita M, Somasundaram A & Prakriya M 2011 Competitive modulation of Ca^{2+} release-activated Ca^{2+} channel gating by STIM1 and 2-aminoethylphenyl borate. *Journal of Biological Chemistry* **286** 9429–9442. (doi:10.1074/jbc.M110.189035)
- Yule DI, Betzenhauser MJ & Joseph SK 2010 Linking structure to function: recent lessons from inositol 1,4,5-trisphosphate receptor mutagenesis. *Cell Calcium* **47** 469–479. (doi:10.1016/j.ceca.2010.04.005)
- Zeng XH, Navarro B, Xia XM, Clapham DE & Lingle CJ 2013 Simultaneous knockout of Slo3 and CatSper1 abolishes all alkalization- and voltage-activated current in mouse spermatozoa. *Journal of General Physiology* **142** 305–313. (doi:10.1085/jgp.201311011)
- Zhang Y, Malekpour M, Al-Madani N, Kahrizi K, Zanganeh M, Mohseni M, Mojahedi F, Daneshi A, Najmabadi H & Smith RJ 2009 Sensorineural deafness and male infertility: a contiguous gene deletion syndrome. *BMJ Case Reports*. pii: bcr08.2008.0645. (doi:10.1136/bcr.08.2008.0645)
- Zhao C, Guo XJ, Shi ZH, Wang FQ, Huang XY, Huo R, Zhu H, Wang XR, Liu JY, Zhou ZM *et al.* 2009 Role of translation by mitochondrial-type ribosomes during sperm capacitation: an analysis based on a proteomic approach. *Proteomics* **9** 1385–1399. (doi:10.1002/pmic.200800353)
- Zittrams N, Borth H, Ackermann F, Meyer D, Vieweg L, Breit A, Gudermann T & Boekhoff I 2010 The 'acrosomal synapse' subcellular organization by lipid rafts and scaffolding proteins exhibits high similarities in neurons and mammalian spermatozoa. *Communicative & Integrative Biology* **3** 513–521. (doi:10.4161/cib.3.6.13137)

Received 9 March 2015

First decision 18 April 2015

Revised manuscript received 22 April 2015

Accepted 11 May 2015